THE CHEMISTRY OF ENAMEL CARIES

C. Robinson*
R.C. Shore
S.J. Brookes
S. Strafford
S.R. Wood
J. Kirkham

University of Leeds, Leeds Dental Institute, Division of Oral Biology, Clarendon Way, Leeds LS2 9LU, United Kingdom; *corresponding author, orl6cr@oralbio.novell.Leeds.ac.uk

ABSTRACT: The chemical changes which occur during the process of carious destruction of enamel are complex due to a number of factors. First, substituted hydroxyapatite, the main component of dental enamel, can behave in a very complex manner during dissolution. This is due not only to its ability to accept substituent ions but also to the wide range of calcium phosphate species which can form following dissolution. In addition, the composition, *i.e.*, the extent of substitution, changes throughout enamel in the direction of carious attack, *i.e.*, from surface to interior. Both surface and positively birefringent zones of the lesion clearly illustrate that carious destruction is not simple dissolution. Selective dissolution of soluble minerals occurs, and there is the probability of reprecipitation. The role of fluoride here is crucial in that not only does it protect enamel *per se* but also its presence in solution means that rather insoluble fluoridated species can form very easily, encouraging redeposition. The role of organic material clearly needs further investigation, but there is the real possibility of both inhibition of repair and facilitation of redeposition. For the future, *delivering* fluoride deep into the lesion would appear to offer the prospect of improved repair. This would entail a delivery vehicle which solved the problem of fluoride uptake by apatite at the tooth surface. Elucidation of the role of organic material may also reveal putative mechanisms for encouraging repair and/or protecting the enamel mineral.

Key words. Caries, chemistry, enamel, microstructure.

Introduction

Dental caries is perhaps the most ubiquitous disease that has afflicted mankind. While it is not normally a fatal condition, it can cause a great deal of pain and distress, and the loss of teeth has profound consequences in terms of eating, speaking, and social behavior in general. The prevalence of the disease also means that the worldwide financial cost of treating the disease is enormous.

Dental caries, viewed simply, is the destruction/demineralization of the tooth's calcified tissues by acid generated in oral "plaque biofilms". This process usually begins with demineralization of enamel and proceeds to the underlying dentin and finally the pulp. Cemental tissue may also be involved if the tooth root is exposed to the oral environment.

There are several features of dental caries which render the disease unique:

First, enamel caries in particular, with which this review will deal, can be regarded almost exclusively as a chemical process which, since enamel is entirely acellular, can be considered to occur without the participation of host cells. As a result of this, many of the preventive and reparative strategies can be self-activated *via* shifts in the local chemical environment.

Second, the acid assault on the tooth is episodic, with destructive episodes occurring more or less continually. Reparative measures are therefore needed constantly, *i.e.*, the environment needs to be continuously monitored and adjusted to sustain the natural repair (remineralization) process. Thus, while it is usually convenient to discuss the chemical changes associated with caries in a "snapshot" fashion–and indeed almost all data concerning chemical changes are collected in this way–the process is entirely dynamic. Even with little in the way of external acid challenge, for example, more stable enamel crystal surfaces will inevitably tend to emerge as recrystallization of the enamel mineral phase occurs with time.

While there has been a great deal of work directed toward our understanding of enamel caries, progress has been slow. The main reasons for this are the very small size of the tissue (which has led to pooling of samples for analysis), the complex structure (which is intimately

associated with chemical composition at the histological level), and the extreme hardness of the tissue (which makes sampling of the tissue at all levels of resolution, from whole tooth down to the histological level, extremely difficult). While much data has been obtained from studies of hydroxyapatite mineral and pooled enamel, most pertinent data in terms of the tissue have emerged from micro-sampling based on mechanical and/or chemical procedures.

The following review attempts to describe the chemical changes which occur during carious destruction of tooth enamel. These changes are considered in terms of the intimate relationship between enamel's complex microstructure and its chemical composition.

Enamel Microstructure

Enamel is an acellular tissue comprised 80-90% by volume of crystals of carbonated calcium hydroxyapatite (Angmar *et al.*, 1963; Robinson *et al.*, 1971, 1983; Elliott, 1997). The remaining 10-20% consists of fluid and organic, usually proteinaceous, material. The distribution of these components is not homogeneous (Angmar *et al.*, 1963; Robinson *et al.*, 1971, 1983), being for the most part related to specific tooth morphology.

The carbonated-apatite crystals are long (possibly up to 1 mm), 50 nm wide by 25 nm thick, extending from the dentin toward the enamel surface (Johansen, 1965). It is thought that they may actually extend unbroken from dentin to the enamel surface. They are arranged in bundles of approximately 1000 crystals, the so-called enamel prisms. The cross-sectional profile of the prisms varies from circular to keyhole-shaped. The hydroxyapatite crystals are primarily arranged with their long (c-) axes parallel to the long axes of the prisms. At the periphery of each prism, however, the crystals deviate somewhat from this orientation, producing an interface between prisms where there tends to be more intercrystalline space (Boyde, 1989). Such space is likely to offer diffusion pathways within the tissue, an important feature with regard to caries. Interprismatic crystals may exist as separate structures, but it is often difficult to distinguish these from the tails of adjacent prisms.

The density of crystals/prisms throughout the enamel, which determines mineral content, is not uniform. In general, this decreases from the tissue surface toward the dentin, while (presumably) porosity, fluid, and organic material increase in this direction. In specific locations, however, the porosity, protein, and crystal distribution may be quite complex (Robinson *et al.*, 1971, 1983). For example, fissure enamel has a very complicated prismatic structure. The rather low mineral and high protein content, indicative of more porosity, is probably due to poorer prismatic packing (Robinson *et al.*, 1983).

Hydroxyapatite Crystal Structure

Since enamel is comprised 80-90% of carbonated hydroxyapatite, the structure of this mineral is important in terms of our understanding how the tissue behaves when subjected to acid dissolution. The mineral component of enamel is basically a substituted calcium hydroxyapatite, the stoichiometric formula for hydroxyapatite being $Ca_{10}(PO_4)_6(OH)_2$ (Kay *et al.*, 1964).

The stoichiometric structure is most easily appreciated by a consideration of the arrangement of ions around the central hydroxyl column, which extends in the c-axis direction through the long axes of the crystals (see Robinson *et al.*, 1995a). In the plane of the diagram, the hydroxyl ion is enclosed by a triangle of calcium ions (calcium II). This in turn is surrounded by a triangle of phosphate ions rotated out of phase by 60°. These triangles are in turn surrounded by a hexagon of calcium ions (calcium I). The entire crystal structure can be envisaged as a series of such hexagonal plates stacked one on top of another, each rotated 60° in relation to its immediate neighbors (Fig. 1). Fig. 2 shows this structure in relation to the crystallographic unit cell (Ichijo *et al.*, 1992).

However, apatite in enamel, and indeed in all other mineralized tissues, exhibits a number of variations on this theme. Such variations include missing ions, particularly calcium (Posner and Perloff, 1957; Winand *et al.*, 1961) and hydroxyl (Young and Spooner, 1969; Myrberg, 1968). Hydroxyl was reported as being 20-30% lower in enamel compared with stoichiometric apatite. Extraneous ions such as carbonate, fluoride, sodium, and magnesium are also frequently found within the crystal structure. A more realistic stoichiometry based on chemical analysis would be: $[Ca]_{10-x-y}[HPO_4]_v[PO_4]_{6-x}[CO_3]_w[OH]_{2-x-y}$, where v+w = x (from Kuhl and Nebergall, 1963). As is indicated by the stoichiometry below, carbonate and acid phosphate groups are present in appreciable amounts. Fluoride will also replace hydroxyl to some extent (Young, 1975). Average compositions for enamel apatite have been calculated at: $Ca_{9.48}Mg_{0.18}Na_{0.11}(PO_4)_{5.67}(CO_3)_{0.45}(OH)_{1.54}(H_2O)_{0.46}$ (from Hendricks and Hill, 1942) and $Ca_{8.68}(HPO_4]_{0.16}[CO_3]_{0.54}[PO_4]_{5.26}[OH]_{0.1}$ (from Moreno and Aoba, 1990).

Such defects and substitutions do have a profound effect on the behavior of apatite, especially with regard to its solubility at low pH. It has been reported that the solubility product for enamel mineral, for example, is higher than that calculated for stoichiometric apatite. Solubility product values for enamel ranged from 7.2×10^{-53} to 6.4×10^{-58} (Patel and Brown, 1975), compared with that for stoichiometric hydroxyapatite of 3.04×10^{-59} (McDowell *et al.*, 1977). Working values are usually taken from this range, and Margolis and Moreno (1985) have used 5.5×10^{-55} as

a working estimate. These rather high values are almost certainly due to the defective nature of the lattice and the inclusion of impurities such as carbonate, magnesium, and possibly sodium.

An appreciation of these values and the effects of substituents on them is also of profound importance when one is studying the solution concentrations required for redepositing enamel mineral which has been dissolved by plaque acids.

Incorporation of Extraneous Ions into Enamel Apatite

(A) Fluoride

Fluoride incorporation is classically thought to occur by fluoride ions filling hydroxyl vacancies in the c-axis columns or displacing hydroxyl ions (Kay *et al.*,1964; Young, 1975). The high charge density on the fluoride ion, together with its symmetry, leads to a much closer fit for fluoride within the Ca II triangles. This has the effect of lowering lattice energy and effectively stabilizing the crystal structure. This resulting solubility product (Ksp) for fluoridated mineral is lowered, rendering it more difficult to dissolve fluoridated crystals and making it easier, *e.g.*, with lower solution concentrations, to redeposit fluoridated crystals. This behavior is of crucial importance to the role of fluoride in dental caries prevention/control.

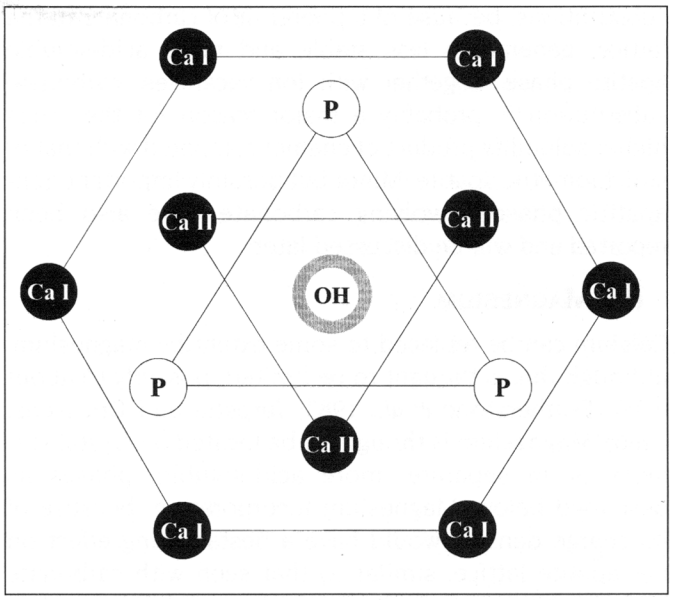

Figure 1. Crystal structure of hydroxyapatite: the overall planar hexagonal nature of the arrangement of calcium and phosphate ions around the central c-axis hydroxyl column can be seen.

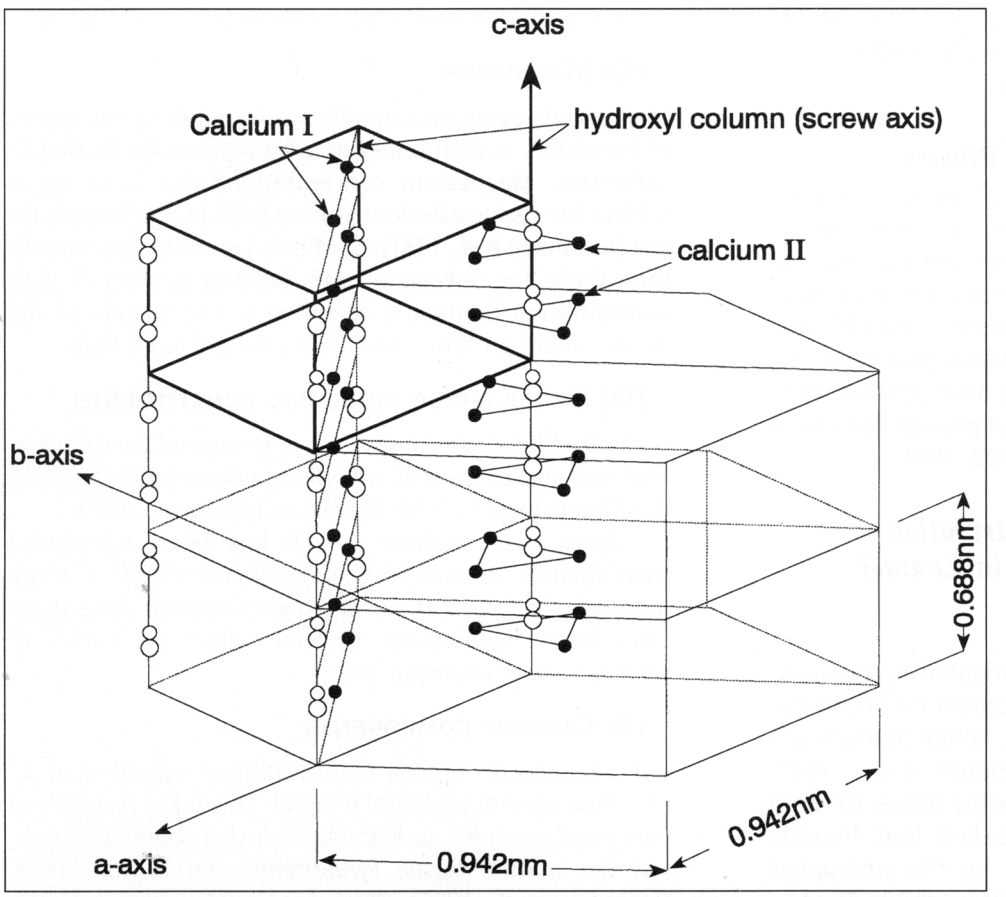

Figure 2. Crystal structure of hydroxyapatite: relationship between hexagonal unit structure shown in Fig. 1 and the rhomboidal crystallographic unit cell (shown in heavier lines) (from Ichijo *et al.*, 1992).

(B) Carbonate

Carbonate can replace either hydroxyl (type A) (Elliott *et al.*, 1985) or phosphate/acid phosphate (type B) (LeGeros, 1983). This depends upon local pCO_2 during crystal development. Substitution of carbonate for phosphate is also thought to involve sodium for calcium exchange. Sodium in this way would reflect carbonate concentrations. The suggestion has also been made that the centers of the crystals may be less well-ordered and accommodate carbonate as a result of (or even responsible for) screw dislocations in the direction of the c-axis (Daculsi and Kerebel, 1977; Marshall and Lawless, 1981). These

substitutions, because of a poorer fit of carbonate in the lattice, generate a less stable and more acid-soluble apatite phase. Together with ion vacancies, carbonate substitution is probably a major reason for the much higher solubility product of enamel compared with that of stoichiometric apatite. Minor but possibly important non-apatitic phases involving carbonate have also been reported and will be discussed later.

(C) Magnesium

Calcium can be replaced to some extent by magnesium, although this is thought to be limited, possibly to about 0.3% (Featherstone *et al.*, 1983; Terpstra and Driessens, 1986). Magnesium is thought to be located on crystal surfaces or in separate, more acid-soluble, phases as described below. Magnesium incorporation, because of its charge density, would have a destabilizing effect on the apatite lattice, similar to that seen with carbonate and would, as a result, raise the solubility product required for precipitating phases.

In addition, it should also be borne in mind that carbonate and magnesium also have a positive synergistic effect, both on their incorporation by the hydroxyapatite lattice and in their ability to increase the acid solubility of apatite mineral (LeGeros, 1984). In this context, it is useful to consider these ions together.

Non-apatitic Mineral Phases

While the bulk of the enamel mineral phase is considered to be substituted hydroxyapatite as described above, there have been suggestions that other separate mineral phases are present, particularly in relation to both magnesium and carbonate. These may be concentrated at the crystal surfaces or at the interface between prisms, a possible result of recrystallization during development. Both Ca $Mg(CO_3)_2$ and $Ca_9Mg(PO_4)_6(HPO_4)$ phases have been proposed (Driessens and Verbeek, 1982, 1985).

Concentration and Distribution of Extraneous Materials in Enamel

(A) Fluoride

The distribution of fluoride (*i.e.*, fluoridated hydroxyapatite) is not homogeneous. Fluoride content is concentrated very much at the enamel surface, falling dramatically toward the tissue interior (Weatherell *et al.*, 1972; Robinson *et al.*, 1983). At the surface of the tissue, the high concentration probably reflects the fact that fluoride accumulates, during development, by the unerupted enamel scavenging fluoride ion from tissue fluids. The formation of a more stable fluoridated mineral at the enamel surface would effectively mop up any fluoride entering the tissue, thereby restricting the passage of fluoride ion to the deeper layers of tissue. Importantly, this phenomenon can also occur post-eruptively in the oral environment in stagnation (caries prone) sites protected from wear. In most other sites, wear removes fluoride from the outer surfaces (Weatherell *et al.*, 1972). This is not to say that fluoridated apatite is the only form of fluoride at the enamel surface. Fluoride-containing components reported at the enamel surface range from ill-defined fluoride phosphate complexes (Christofferson *et al.*, 1988) through calcium fluoride (Nelson *et al.*, 1984; Rølla and Øgaard, 1986) to the extended calcium fluoride hydrogen bonded complexes described by Kreinbrink *et al.* (1990). All, however, place high concentrations of fluoride ion at the surface of the tooth, where carious attack is initiated.

(B) Carbonate

Incorporation of carbonate, unlike fluoride, seems to occur exclusively during tissue development, presumably reflecting the pCO_2 in the immediate environment of the crystals. Concentrations rise from the enamel surface from about 2% toward the dentin, where concentrations of 4-6% are found. The gradient is often interrupted by pockets of relatively high concentrations, for example, in fissure enamel in molar teeth (Weatherell *et al.*,1968a).

(C) Magnesium

Magnesium, again presumably incorporated during enamel formation, is distributed in a fashion similar to that of carbonate. Magnesium concentrations rise from about 0.2% at the tissue exterior to about 0.5% in the tissue interior (Robinson *et al.*, 1981). Gradients are often less smooth than those for carbonate, with isolated pockets of high concentration frequently occurring in the vicinity of the dentin where protein concentrations tend to be high.

(D) Other minor inorganic constituents

Sodium, chloride, and even minor amounts of, for example, lead may also be present, with distributions similar to those described above (see Weatherell and Robinson, 1973).

Apart from sodium, which has been associated with apatite destabilization (Verbeek 1986), a more detailed account of these has been omitted, since there have been few reports of their effect on caries at endogenous concentrations.

(E) Organic components

While usually at very low concentrations, organic material is also present in dental enamel. This takes the form of very small peptides and amino acids distributed throughout the mature tissue (Weidmann and Hamm, 1965; Robinson *et al.*, 1975). This presumably represents the remnants of the original developmental matrix, perhaps retained by binding to the hydroxyapatite crystals. However, an insoluble protein material is also present,

most frequently related to the enamel tufts, in highest concentrations near the dentin and in areas where crystal packing is less compact-for example, the cusps and fissure regions (Weatherell *et al.*, 1968b; Robinson *et al.*, 1975, 1999). This is of unknown composition, although the presence of amelin (ameloblastin/sheathelin) in tuft protein has been reported recently (Robinson *et al.*, 2000).

Carious Attack, Histological Changes

(A) Pathway of attack in relation to enamel micro-architecture

Histological examination of caries lesions of enamel has consistently suggested that the earliest mineral, *i.e.*, the most accessible and/or most soluble material, is removed from the periphery of the prisms (Darling, 1961). Whether this represents preferential dissolution of crystal surfaces or dissolution of a separate mineral phase (Driessens and Verbeek, 1982, 1985) is not known. What is likely, however, is that it reflects the lower crystal packing seen at this site, permitting easier diffusion of acids and protons into the tissue and mineral ions out of it. Subsequent dissolution then appears to track across the prisms at the cross-striations, followed by dissolution of the prism bodies.

(B) Pore structure of the caries lesion

Perhaps the most useful, or at least the most thought-provoking, approach to the study of structural changes associated with caries lesions was that which used polarized light and imbibition media (Darling, 1956, 1961). Essentially, this approach was able to demonstrate a complex changing pore structure as the lesion developed, related to the structural pathways described above. More importantly, it has been possible for these changes in pore structure to be related to specific alterations in tissue chemistry.

(C) Lesion zones

Four porosity-related zones were described in the caries lesion (Darling 1956, 1961). These are described below in the order in which they would appear in traveling from sound enamel to the enamel surface (Fig. 3A).

(i) Translucent zone

The first visible carious change in the enamel, corresponding to a loss of about 1-2% mineral, was composed of a small number of relatively large pores (Fig. 3A). These are of a size which would admit molecules such as 2-chloronaphthalene or quinoline. Such materials were chosen because, at the same refractive index as enamel, they rendered the tissue translucent. Initial interpretations suggested that the first step was protein removal, followed by loss of inorganic ions. While mineral loss has been shown, loss of organic material has not been convincingly demonstrated. Much of this first loss also appeared to derive from interprismatic and intercrystalline regions (Fig. 3E), due in part to an easier flux of ions through these regions (Darling, 1961; Arends and ten Cate, 1981).

(ii) Positively birefringent (dark) zone

The succeeding stage appeared to be positively birefringent (Darling, 1961), apparently containing, in addition to the larger pores of the translucent zone, smaller pores which did not admit chloronaphthalene (Poole *et al.*, 1961). Porosity had increased to 5-10% (Fig. 3A). The additional small pores were puzzling and were thought to be due to removal of small mineral domains. However, an alternative explanation for the generation of smaller pores was the possibility that occlusion of some of the larger spaces in the initial translucent zone had occurred (Silverstone, 1967) (Fig. 3E). It was suggested that this represented some remineralization, raising the concept of a natural repair process. More recently, however, it has been suggested that such occlusion might also be due to redistribution of endogenous organic material or accumulation of exogenous protein (Robinson *et al.*, 1998). The implication of this with regard to lesion progress is discussed later.

(iii) Body of the lesion

Further demineralization produces the body of the lesion (25-50% porosity), the pores of which enlarge until mechanical destruction of the tissue, *i.e.*, cavitation, occurs (Figs. 3A, 3E).

(iv) Surface zone

Shortly after the initial attack (Darling, 1956, 1961), recognized as a translucent zone (Darling, 1961; Silverstone, 1968; Margolis *et al.*, 1999), an apparently relatively "intact" surface zone develops. Subsequent demineralization then proceeds in the immediate sub-adjacent tissue. Porosity of this surface zone amounts to 1-2%, which is fairly close to that of sound tissue. This zone often persists until cavitation occurs.

Chemical Changes in Enamel during Carious Attack

A great deal of important data has been reported concerning the detailed physical chemistry of enamel dissolution based on both kinetic and thermodynamic approaches to enamel and apatite dissolution. As indicated above, however, enamel is neither structurally nor chemically homogeneous. Both structural and chemical gradients exist within the tissue, often extending from the surface toward the dentin. It is essential to bear these in mind when discussing chemical changes during carious attack.

The structure of enamel, especially its microporosity, no doubt affects the diffusion of materials

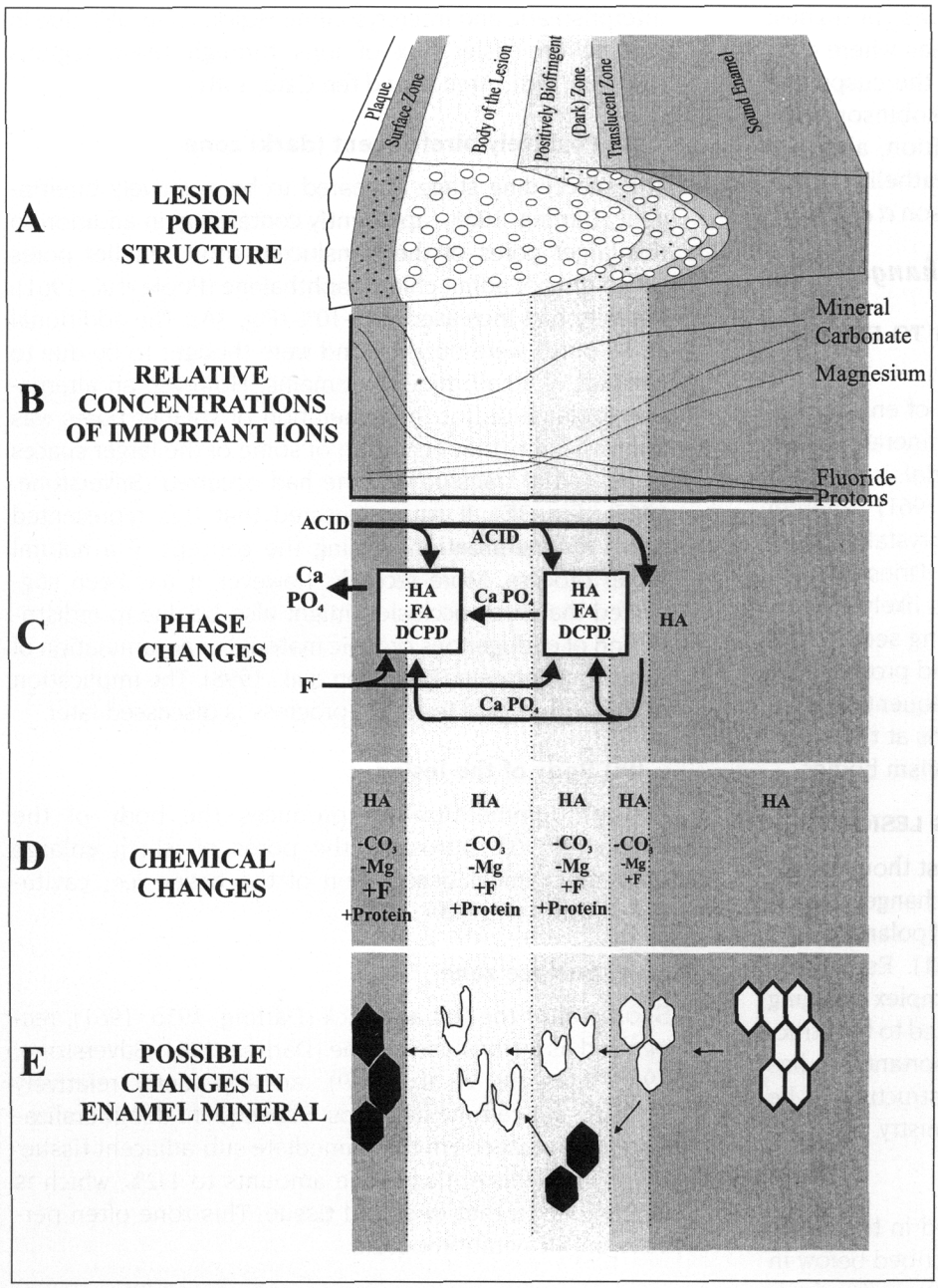

Figure 3. Diagrammatic summary of the changes within the zones of a caries lesion. (A) Relative pore structure of the four zones of a caries lesion of enamel. (B) Relative concentrations of important ions at each stage of carious attack. Selective loss of magnesium and carbonate is illustrated together with concentration gradients of fluoride and protons from surface to interior. (C) Proposed phase changes in the surface zone and the positively birefringent zone following ingress of protons and fluoride and a net loss of mineral. (D) Net chemical changes detected at each stage of carious attack. (E) Diagrammatic representation of changes in enamel mineral crystal morphology within each zone to account for changes in pore structure.

both into and out of the tissue. The low diffusion constants for enamel (Burke and Moreno, 1975; Borggreven *et al.*, 1981) are thought to be mainly a reflection of the small sizes of these pores, the most important of which seem to be situated at prism boundaries (Boyde, 1989). Changes in pore structure, which will be generally an increase as mineral is removed, will enhance entry and egress of materials. On the basis of the fact that fluid within caries lesions was saturated with respect to hydroxyapatite, it was suggested that diffusion might be rate-limiting (Vogel *et al.*, 1988). On the other hand, *in vitro* studies showing a near-linear increase of lesion depth with time contradicted this view (Chow and Takagi, 1989). This, however, will be complicated by the fact that pore reduction, by redeposition in positively birefringent and surface zones, together with the chemical changes described below, is likely to hinder demineralization.

With regard to the variations in the chemical composition of enamel, this has profound implications with regard to the kinetics of demineralization and remineralization. However, much of this work has been carried out with the use of hydroxyapatite and enamel powder or, at best, enamel sections. Analysis of these kinds of data suggests that demineralization may, to a large extent, be surface-controlled (Margolis *et al.*, 1999). The large variations in enamel composition, including local concentration gradients of specific mineral ions (Weatherell *et al.*, 1968a; Robinson *et al.*, 1971, 1981, 1983) as well as endogenous organic material (Robinson *et al.*, 1983) and organic acids (Gray, 1962; Featherstone and Rodgers, 1981), are therefore likely to result in large local variations in rates of both demineralization and remineralization. General models for enamel caries in terms of chemistry and structure are therefore difficult to design.

In discussing chemical changes during caries, we have therefore endeavored to relate the micro-architectural to the chemical structures of both intact tissue and the caries lesion. In the following sections, we have attempted to draw this information together with that derived from physico-chemical studies of bulk tissue and, where appropriate, hydroxyapatite.

Relationship of Changes in Enamel Pore Structure during Carious Attack to Chemical Change

(A) Surface zone

The mineral content of the surface zone is similar to that of sound enamel (Darling, 1961), implying that it is either protected from dissolution compared with underlying tissue or that it forms/reforms during the caries process. The current consensus view is that, for the most part, it occurs by redeposition of material dissolved from deeper layers, with perhaps some contribution from plaque fluid. The underlying mechanism for surface zone formation, however, is still a matter of some controversy, and several hypotheses have been put forward.

(i) The protective chemistry of the sound enamel surface

The apparent preservation of the surface zone initially suggested that its character *per se* renders it less susceptible to acid attack. It contains, for example, high concentrations of fluoride, which stabilizes apatite (Weatherell *et al.*, 1972), and low carbonate (Weatherell *et al.*,1968a; Robinson *et al.*, 1983) and low magnesium (Robinson *et al.*, 1981), which have a reverse, destabilizing effect. This would favor a lower acid solubility for mineral in this tissue region, effectively protecting it from dissolution. At the same time, penetration of acid into the deeper, more soluble, layers would remove interior mineral in preference to the outer tissue. The outer tissue could then continue to accumulate fluoride and become even more acid-resistant.

The normal presence of organic material on or in the enamel surface (the pellicle) has also been suggested as a contributor to surface zone formation by reducing mineral loss or acting as a permselective barrier (Meckel, 1968; Francis *et al.*, 1973; Zahradnik *et al.*, 1976; Gray, 1977). Subsequent investigations have also shown that natural lesions were able to take up more calcium from the external environment *in vitro* when protein material had been removed (Robinson *et al.*, 1990). This supports the view that protein layers and/or, for example, lipid on or in the enamel surface can slow the transit of mineral ions through the enamel surface and, in doing so, may facilitate the precipitation of mineral in this region.

In the context of dynamic exchange such as that between the enamel surface and plaque, it becomes difficult to study the enamel surface in isolation. While the concept of a resistant surface is not now regarded as an exclusive reason behind surface zone formation, it is likely to contribute toward surface zone formation *in vivo*, although the extent of this contribution is unknown.

(ii) Chemical gradients in enamel

In an expansion of the above argument, moving inward away from the surface, gradients of fluoride decrease, while carbonate and magnesium gradients increase together with increasing porosity (Figs. 3A, 3B) (Weatherell *et al.*, 1968a; Robinson *et al.*,1971, 1981). Therefore, as the caries process tracks inward toward the dentin, the chemistry of dissolution will change, with the tissue showing evidence of increasing solubility (Theuns *et al.*, 1986). This information has been used to model changes in the lesion (van Dijk *et al.*, 1979). No presumptions were made concerning the relative importance of rates of dissolution or rates of diffusion. Chemical gradients were interpreted as gradients in enamel solubility product, likely rate constants for enamel dissolution and increase in porosity. The conclusions supported the view that surface zones could form as a result of acid dissolution along these chemical gradients. The model emphasizes the dissolution rate and a complexation function apparently related to stabilization of the surface. While the original model relates only to the natural surface, it would also apply to stabilizing molecules entering the outer parts of the lesion.

In a consideration of porosity, penetration of undissociated acid and protons into the complex micro-particulate enamel microstructure may also play a role in generating subsurface demineralization. The close packing of crystals during dissolution may affect the kinetics of mineral loss, leading to the formation of a surface zone. Dissolution of ions into the very small intercrystalline volume will tend to produce high solution concentrations and thus generate a high concentration gradient away from the lesion front. This may well tend to accelerate mineral movement away from the advancing edge of the lesion front, explaining the deep penetration of the leading edge of the lesion with a slower removal of mineral from later stages (Robinson *et al.*, 1983).

(iii) Stabilization of the outer enamel layers

Following the argument that endogenous fluoride stabilizes the outer enamel mineral, several reports considered the uptake of stabilizing components from the plaque. Components, including fluoride, which could adsorb to the surface or diffuse in from the plaque/saliva interface and stabilize the enamel could also facilitate the formation of a surface zone. Organic components, mainly proteins from the saliva such as those seen in pellicle, may not only affect transport into and out of the enamel (Robinson *et al.*, 1990) but also, together with components such as fluoride and, for example, pyrophosphates (Francis *et al.*, 1973; Gray, 1977), stabilize the enamel mineral. Fluoride is of particular importance in this respect, since it produces a less-acid-soluble mineral. However, the role of fluoride in this context

may be rather more involved than simple solubility. While it is clear that fluoride provides less soluble apatite and will facilitate redeposition, it will also facilitate the hydrolysis of acidic calcium phosphate phases such as dicalcium phosphate dihydrate (DCPD) and octocalcium phosphate (OCP) to the more stable fluoridated apatite (LeGeros, 1991). In addition, the presence of other phases such as calcium fluoride, derived from fluoride in the plaque fluid, may be important (ten Cate and Duijsters, 1983). It was suggested that calcium fluoride might not only form a reservoir of fluoride but also may offer a more effective diffusion barrier than fluoridated apatite at the tooth surface.

Discriminating between the effects of rendering enamel mineral less acid-soluble and facilitating redeposition is clearly difficult. Perhaps all that can be said at present is that some *in vivo* studies have shown greater effects on inhibition of demineralization than on remineralization. This may be complex, however, in that at any pH capable of dissolving enamel, fluoride in solution would seem to offer some protection (ten Cate and Duijsters, 1983). For fluoride-stimulated remineralization (Koulourides *et al.*, 1961; von der Fehr *et al.*, 1970), the situation is less straightforward. Fluoridated mineral will have a lower solubility product and will tend to precipitate readily, mainly at the surface. If blocking of surface porosity occurs, the repair process would be restricted to the surface layer. In this sense, fluoride could be said to be less effective at facilitating remineralization than inhibiting demineralization, since it would not lead to repair deep within the lesion.

Clearly, interplay between the enamel surface and the immediate environment is crucially important, and it may not be possible, as suggested above, to distinguish between protection effected by endogenous properties and that afforded by extraneous components.

(iv) Dissolution and precipitation phenomena

The three general approaches described above to account for the formation of an apparently intact surface zone are based on an appreciation of the chemical and physical properties of the tissue as well as the immediate natural environment of the tooth surface. An important experiment (Langdon *et al.*, 1980), however, demonstrated that a further mechanism might need to be considered. Using compressed pellets of hydroxyapatite under acidic conditions, they demonstrated that an apparently intact surface zone could be generated in an acid gel containing 2 ppm fluoride. This occurred without a unique surface chemistry, without any chemical gradients, and without the adsorption of extraneous material from the environment. It must be pointed out, however, that, in their solution, they did use 2 ppm fluoride, which could, by adsorption, have produced a less soluble surface mineral or affected supersaturation levels required to precipitate fluoridated mineral. In addition, the microparticulate structure of the compressed pellet may contribute to surface zone formation, as described above for enamel. Somewhat similar results were obtained by the formation of surface zones on abraded enamel, where at least the immediate surface chemistry had been removed (Silverstone, 1968).

Nonetheless, sufficient concern was raised that further mechanisms were sought which might explain surface zone generation in the absence of chemical gradients, adsorbed materials, or porosity variations.

One approach, also involving compressed pellets of hydroxyapatite, proposed a coupled diffusion model for the generation of surface zones (Anderson and Elliott, 1987; Gregory *et al.*, 1991). Coupled diffusion is particularly important when acids are able to diffuse into salt solutions. This situation is analogous to caries lesion formation, where the plaque biofilm compartment is considered to produce acid continuously, and the solution within the lesion is in equilibrium with the enamel mineral. The composition of this compartment will change as ions enter or leave it. The mechanism for ion transfer is related to the high mobility of protons due to "tunneling". By rapid movement into the enamel, protons can set up an electric field (diffusion potential) across the enamel surface into the underlying tissue. Movement of dissolved ions from the enamel will depend on this potential as well as on their own concentration gradient. If the diffusion potential is large enough, a build-up of dissolved ions from enamel can occur, even against a concentration gradient, and could result in the formation of a surface layer.

A more extensively reported approach considered only the diffusion of components into and out of the surface zone of the tissue, together with dissolution and precipitation phenomena.

The approach requires that dissolved material entering the surface zone from the tissue interior must do so more rapidly than material leaving the surface zone for the plaque, in order that some net precipitation can occur. One factor may be the retention of calcium and phosphate in the enamel surface by binding to the hydroxyapatite. Early work in this area (Moreno and Zahradnik, 1974) proposed that the chemical potential of phosphoric and organic acids (from plaque bacteria) will be higher in the outer part of the lesion and will, therefore, drive inward and will eventually be neutralized. The reverse would be true for basic components such as calcium and hydroxyl ion.

The experimental approach used powdered enamel in "equilibrium" experiments to establish solution conditions at a variety of pH and [F] values. Whole teeth were also used to verify the presence (or not) of a surface zone. Comparison of these data with theoretical solubility calculations raised the possibility that some phase transformations could occur in the enamel surface under the conditions used. On this basis, Moreno and Zahradnik (1974) proposed that, following

TABLE

Chemical Composition of Mineral LOST from Each Zone of Caries Lesions of Human Enamel Compared with the Composition of Sound Enamel (from Robinson *et al.*, 1983, p. 209)

	Sound Enamel	Lost from Translucent Zone	Lost from Positively Birefringent (dark) Zone	Lost from Body of the Lesion
Calcium	37%	≈ 30%	≈ 35%	37%
Phosphorus	18.5%	≈ 13%	≈ 16.6%	18.5%
Carbonate	2-4%	≈ 28%	≈ 3%	≈ 1.0%
Magnesium	0.2-0.4%	≈ 2%	≈ 3%	≈ 0.16%

some enamel surface demineralization, precipitation of some $CaHPO_4.2H_2O$ (DCPD) and $Ca_5F(PO_4)_3$ (FA) occurred (Fig. 3C). The composition of the supernatant solution would reflect initial pH, acid concentration, and the thermodynamic properties of the three phases. Calculated values for this solution compared favorably with experimental results from equilibration experiments. The data were amplified in later work (Margolis and Moreno, 1985), where plots of chemical potential were used to describe solution conditions. At low pH values (4.3 and 5.5 in this case), solubility curves crossed enamel and DCPD solubility lines at or above the singular point, indicating the probability of the phase changes suggested (Fig. 4). To maintain the equilibrium between these phases at the singular point, as mineral ions enter the surface zone from deeper within the tissue, Moreno and Margolis proposed that proportional amounts of DCPD and apatite would precipitate (Fig. 3C). In a later study (Margolis *et al.*, 1999), these data was verified. In addition, it was noted that the specific organic acids present could also affect demineralization rates, lactate being more effective than acetate or propionate. The reasons for this are not clear, but may relate to differences in the ability of acid anions to bind to the mineral surfaces. This is a complicated factor, since plaque-generated acid is likely to be a complex mixture such that this aspect of dissolution may not be amenable to investigation *via* a predictive model.

While it has been difficult to demonstrate the predicted specific mineral phases that may develop in the surface zone, some transformation was supported by structural data. Greater crystallinity in the surfaces of enamel caries lesions has been reported (Johnson, 1967), together with increased apatite crystal diameters in the surface zones of natural and artificial lesions (Silverstone, 1983).

The essentials of this approach have highlighted the fact that the kinetics of net dissolution and the behavior of the surface zone are driven by levels of super- or undersaturation with regard to the mineral phases concerned. The surface zone therefore represents a constantly dissolving and re-forming layer of several mineral species.

While this generalized approach is a reasonable mechanism for surface zone formation, the variations in enamel chemistry–possibly including the presence of non-apatitic, carbonate-, and magnesium-rich phases at the histological level–must raise questions concerning local effects. For example, initial dissolution of the enamel surface prior to surface zone appearance almost certainly removes carbonate and magnesium selectively, while leaving behind fluoride-enriched tissue. This was supported in equilibration experiments (Shellis *et al.*, 1993) where Mg- and Na-enriched fractions were lost preferentially. Solubility products for the remainder were at the higher end of the values previously reported (Patel and Brown, 1975). The nature of the dissolving phases and those remaining must therefore be very different.

There is support for this in the disparity between thermodynamic solubility products for apatite [3.04×10^{-59} (McDowell *et al.*, 1977)] compared with the rather varied solubility product values for enamel 7.2×10^{-53} to 6.4×10^{-58} (Patel and Brown, 1975). As noted, such differences are almost certainly due to the defect nature of the biological lattice and its substituents.

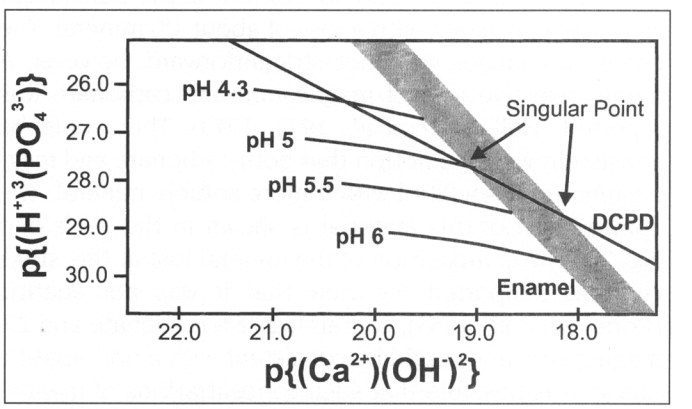

Figure 4. Solubility diagram illustrating proposed model for formation of relatively intact surface zones during enamel caries. The broad zone for enamel indicates the range of reported solubility values (see Margolis *et al.*, 1999).

The model must also take into account that the proposed equilibrium between calcium phosphate phases in the enamel surface and those in surrounding fluid requires not only equilibrium between dissolving normal enamel crystals and solution but also between solution and crystals exhibiting adsorbed ions on the dissolving surfaces. At pH 5, for example, most phosphate present will be in the form HPO_4^{2-}, which will presumably be evenly distributed over the surfaces of the crystals. There is in fact good evidence for the presence of increased HPO_4^{2-} in caries lesions compared with sound enamel (Arends and Davidson, 1975; Brown *et al.*, 1975). It might be difficult, therefore, to separate equilibria with such surfaces from DCPD, especially since estimates for DCPD concentrations are reported at about 0.2% (Margolis and Moreno, 1985).

The value of this approach lies in the fact that it offers an explanation which does not require a unique surface chemistry or chemical gradients. The authors themselves have pointed out, however, that for the situation *in vivo* this is unlikely to be an exclusive explanation for surface zone formation.

(B) Translucent zone

Considered to be the first discernible change in caries, probably preceding the formation of a surface layer, the translucent zone corresponds to a loss of about 1% of mineral. It is worth reiterating that this represents a dynamic situation and that the transitions from sound enamel to translucent zone and from translucent zone to the next stage, *i.e.*, positively birefringent zone, are likely to be in dynamic equilibrium.

From a physico-chemical point of view, much less work has been carried out on these zones. However, an elegant microdissection approach (Hallsworth *et al.*, 1972, 1973) revealed important information.

Mineral content, carbonate content, and magnesium content were determined in the translucent zone. It became clear that the mineral content of the translucent zone was consistent with a loss of about 1% mineral. The chemical changes were not straightforward, however. A highly selective loss of magnesium and carbonate was reported (Hallsworth *et al.*, 1972, 1973). This would be consistent with the notion that both carbonate and magnesium are associated with a more soluble mineral. The composition of this material is shown in the Table and Fig. 3D. The composition of the mineral lost at this stage certainly supported the view that it was not apatitic (Robinson *et al.*, 1983), containing 28% carbonate and 2% magnesium, and would be consistent with a non-apatitic phase. It is possible that such concentrations of magnesium and carbonate are mainly associated with crystal surfaces as a result of selective crystallization during crystal formation. It has also been suggested, however, that some of the carbonate resides at the crystal centers (Marshall and Lawless, 1981), which are also selectively removed during carious dissolution (Johnson, 1967). Alternatively, the composition of material lost would be consistent with separate phases such as dolomite or whitlockite (Driessens and Verbeek, 1982, 1985). While the precise nature of this mineral is not clear, the location of initial mineral loss seems to be at the prism peripheries, perhaps related to ease of access of incoming acids/protons. This raises an interesting question related to the effect of the changing nature of the "supernatant" solution on the supersaturation levels with regard to putative precipitating species. This has considerable implications for formation of both surface zones and positively birefringent zones (see below).

(C) Positively birefringent zone (and surface zone revisited)

Since the translucent zone precedes both the initial surface zone and the positively birefringent zone, it is likely that the chemical changes noted above might, in part at least, explain the initial formation of one and the ongoing formation of the other. Initial loss from the enamel surface of components rich in magnesium and carbonate would render the undissolved tissue much less soluble in acid. Their removal from the supernatant by diffusion out of the enamel surface would shift the equilibrium toward a more easily precipitated mineral (*i.e.*, with lower Ksp values). This would be exacerbated by the uptake of fluoride in the immediate surface zone (Weatherell *et al.*, 1977). This is an important point, since it has been established that dissolution and precipitation of calcium phosphates and enamel are highly dependent on whether the supernatant solution is supersaturated or undersaturated with regard to the forming or dissolving mineral phase (Margolis *et al.*, 1999). Redeposition of a carbonate- and magnesium-depleted and fluoride-enriched mineral, presumably with lower Ksp values, then becomes much more likely (Fig. 3C). Since fluoride will continue to be accumulated in this region, the surface might be expected to become steadily more resistant to acid attack, while deeper enamel continues to lose mineral, which, when transported back to the surface, would tend to reprecipitate. The increasing fluoride would also tend to facilitate hydrolysis of acid phosphates such as DCPD to more stable fluoridated apatites (LeGeros, 1991). This would be similar to the proposed effects of chemical gradients in the tissue (van Dijk *et al.*, 1979).

In progressing from the translucent to the positively birefringent (dark) zone, more mineral had been lost, in that about 5-10% of mineral appeared to have been removed. This zone too, however, had lost selectively more magnesium and carbonate than could be accounted for by bulk crystallite dissolution (Table) (Fig. 3D). The

material lost in terms of carbonate was similar to bulk enamel, and one could conclude that most of the acid-susceptible mineral determined by high carbonate had already been eliminated. The relative concentration of magnesium lost, however, was still high compared with bulk enamel, and there is the possibility that a magnesium-rich fraction was still being removed at a somewhat later stage compared with carbonate. The reasons for this are not clear, but it implies that carbonate may be a more important destabilizing element than magnesium, or that some reprecipitation of a magnesium-containing fraction occurred. Like the translucent zone, concomitant with loss of carbonate and magnesium, some fluoride had been acquired (Weatherell *et al.*, 1977), presumably from the plaque fluid or following some dissolution, from the enamel surface itself.

The reasons for the appearance of many small pores characteristic of the dark zone are still intriguing. If one assumes the interpretation of the imbibition data to be correct, the opening up of microstructural holes, perhaps mineral domains not previously accessible, is possible, although the reasons for this are not obvious.

A further possibility is the occlusion of some pores by accumulation of organic material either from a redistribution of endogenous protein material or by uptake of proteins from the oral environment. Attempts to verify this by the treatment of sections with ethylene diamine did show a reduction in the width of the dark zone (Robinson *et al.*, 1995b). While this is not in itself conclusive, recent data have showed that proteins are present in caries lesions (Robinson *et al.*, 1998). While there is no evidence as to whether these result from endogenous proteins being redistributed during carious attack or are exogenous, specific molecules such as albumin and some immunoglobulins have been identified.

With regard to the effect of protein on enamel caries, the difficulty is in discerning whether such proteins would inhibit demineralization by protecting or stabilizing crystal surfaces, or would encourage remineralization by providing crystal initiation sites. Recent reports (Robinson *et al.*, 2000) have suggested that tuft protein at least can facilitate crystal growth and may therefore assist in protecting enamel containing tuft protein from net mineral loss. Fissure enamel, for example, contains large amounts of insoluble tuft protein, and it has been suggested that such high-protein enamel in a lesion seems to contain rather more mineral than adjacent tissue (Robinson *et al.*, 1983). On the other hand, the presence of components such as albumin, a well-known inhibitor of crystal growth (Robinson *et al.*, 1989; Garnett and Dieppe, 1990), raises questions as to whether resistance of natural lesions to repair might be due to protein-mediated inhibition of crystal growth (Robinson *et al.*, 1998).

A more intriguing explanation for the appearance of small pores is that some crystal growth or redeposition of mineral had occurred, occluding some of the larger pores of the translucent zone (Silverstone, 1967, 1983).

As with the arguments for the surface zone, the degree of supersaturation within the lesion with regard to dissolving and precipitating mineral phases is a crucial factor in the determination of whether precipitation will occur. Loss of carbonate and magnesium, for example, will lead to the presence of a much less acid-soluble residue, *i.e.*, with a lower solubility product, at the leading edge of the dark zone (Robinson *et al.*, 1983; Shellis *et al.*, 1993) (Fig. 3C). Precipitation of these phases, with lower Ksp values, would therefore be more likely with the progressive loss of these ions. Such reprecipitation will also be facilitated by the increasing amount of fluoride entering the tissue from the enamel surface (Weatherell *et al.*, 1977). This would occur whether crystals were reforming or if new mineral was depositing. It would not explain, however, the eventual disappearance of the dark zone as mineral continued to be removed and the body of the lesions formed. One does not have to look far for an explanation for this, however, since there is presumably a pH (Vogel *et al.*, 1988) and organic acid gradient from enamel surface into the lesion. It is distinctly possible, therefore, that the trailing edge of the dark zone, *i.e.*, the oldest part nearest the enamel surface, is subject to a rather lower pH than the front edge, such that even redeposited (less soluble) material would begin to dissolve. This would generate greater pore volumes in the body of the lesion. The process would accelerate as the lesion progressed into the enamel toward higher concentrations of both carbonate and magnesium.

An important consequence of this concept is that demineralization and remineralization could occur simultaneously in the same lesion. This would reinforce the view of the caries process as a continuously evolving situation characterized by its dynamic nature, the oscillating pH seen *in vivo* serving to swing processes toward or away from demineralization.

Support for crystal growth or at least some redeposition within the lesion has been obtained, in that crystal diameters were larger than normal (Silverstone, 1983). In addition, attempts to remineralize lesions often result in an enlarged positively birefringent zone, especially in the presence of fluoride (Silverstone and Poole, 1968; Poole and Silverstone, 1973).

If this argument is true, then the persistence of the surface zone as opposed to the disappearance of the dark zone requires explanation. This may be answered by the fact that the surface zone takes up very large amounts of fluoride as well as organic material. It may thus be mainly a matter of additional stabilization of the surface zone.

(D) Body of the lesion

This could be considered to comprise the final stage of enamel destruction, since continuous enlargement of the pores of this zone ultimately leads to cavitation.

The chemical composition of material lost from this zone is consistent with this view, in that it resembles that of bulk enamel (Table). It too, however, accumulates fluoride and organic material.

This zone is likely to have been the source of data regarding crystallographic changes in enamel mineral during caries (LeGeros, 1991). These data are consistent with the chemical changes described above. This includes larger apatitic crystals associated with lower magnesium and carbonate and higher fluoride and an increase in "a" axis dimension, which would be consistent with increased concentrations of HPO_4^{2-}. Clearly, a great deal of recrystallization occurs during the caries process.

Oscillating pH Conditions in the Mouth

A final word should perhaps be said about the oscillating conditions of pH to which the enamel is thought to be subjected in the mouth. It was considered that demineralization and remineralization might alternate as pH dropped due to acid production and then rise as acid production stopped and local acid was neutralized (see ten Cate, 1983). A consideration of the chemical scenarios described above, however, makes it much more likely that, within the caries lesions, demineralization and remineralization could occur at the same time in the same lesion. This would depend to a large extent on the Ksp values of the mineral phases dissolving and forming at any one time and in specific locations within the lesion. pH would play a prominent role in this process.

Specific periods of low pH might have other effects, however. Ionic fluoride is known to accumulate at tooth and lesion surfaces (Weatherell *et al.*, 1972, 1977), presumably due to hetero-ionic exchange with apatite hydroxyls, producing a more stable crystal. At low pH, however, undissociated HF forms, which, being un-ionized, would penetrate the lesion more easily. Subsequently, during periods at higher pH, some recrystallization of enamel mineral is likely to produce more stable mineral phases by incorporation of absorbed fluoride and hydrolysis of acid phosphates. The interplay between these situations will decide whether the tooth becomes stable to acid attack or continues forward to ultimate destruction.

Directions for Future Research

It is clear from a consideration of the data available that enamel caries is a complex chemical process. While general principles are becoming clear, it is equally obvious that local conditions within individual mouths and teeth are extremely important. The roles of minor constituents of the enamel mineral are especially crucial to the kinetics of tooth dissolution. Removal of magnesium and carbonate and uptake of fluoride both result in reduced net loss of mineral. We should therefore seek to modify the tooth mineral in such a way as to reduce destabilizing elements (magnesium and carbonate) and elevate fluoride, such that the Ksp for enamel mineral is reduced. Investigators should seek the addition of other ion combinations which would produce perhaps not apatite but a more stable substitute. The intimate association of caries with structure and the possibility of remineralization as the lesion progresses also suggest that it is important to drive fluoride or any stabilizing ion into the lesion as it forms. This would require developing a delivery vehicle which prevented fluoride incorporation into apatite at the tooth surface but encouraged uptake deep within the lesion. Finally, little is known about the precise role of organic materials. Clearly they play a significant role. The design of organic molecules which would protect crystals from dissolution and/or facilitate reprecipitation is a distinct possibility, perhaps mimicking the pellicle proteins of salivary origin or designing totally novel materials with the in-built capacity to generate apatite growth as well as protect existing crystals and perhaps neutralize plaque acid.

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